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Analysis of Pigment Production from Soil *Pseudomonas Putida*

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ABSTRACT

The environment conservation and human safety has created awareness for natural pigment sources. Microbial pigments shows better biodegrability and have numerous applications from food to cosmetics when compared to synthetic pigments. The current study aimed to screen and identify pigment producing microbial isolates especially pseudomonas using pseudomonas isolation agar from garden soil. Various time incubation were provided to optimize its maximum pigment production. During the incubation of P.putida in nutrient broth, for the first four days, there was a slight colour change in the media. The colour change was notable from the sixth day onwards. On the eighth day, it was dark green and continued on until the end of the incubation time. The validity of this experiment was demonstrated by colorimetric observation also. The pigment concentration was low (0.089±0.08) during the second day of colorimetric reading. At the time of the sixth and eighth day of colorimetric observation, the concentration of pigments was gradually increased. The concentration of pigment production was high during the tenth day of optical density observation (0.527±0.16). The colorimetric reading in the subsequent days was more or less the same. The density of the pigments remained intact.

Keywords
Pseudomonas putida, biodegradation, pigments, optical density

INTRODUCTION

Nature produces many colorants from various resources including plants and microorganisms, which are possible alternatives to synthetic dyes and pigments currently employed (Parekh *et al*., 2000). In today's living environment, the impact of natural pigment is rapidly spreading and raising awareness among people. The effects and side effects of chemical colouring agents cannot be listed and they are the important cause of diseases that affect the human species (Kamla*et al*., 2012).

The recent awareness of human safety and environmental conservation has made fresh enthusiasm for natural sources of colors. Natural colorants or dyes derived from flora and fauna are believed to be safe because of the non–toxic, non–carcinogenic,non polluted and biodegradable nature (Joshi *et al*., 2003).Among natural pigments, pigments from microbial sources are potentially good alternatives to synthetic pigments (Dufossel,2006).
Pigments responsible for bright colours are synthesized almost exclusively by bacteria belonging to genera *Pseudomonas, Streptomyces, Nocardia, Sorangium, Brevibacterium, Burkholderia*and*Bacillus*. Among them *Pseudomonas* is most eligible pigment producing agent(Yuodim*et al*., 2002). Realizing the importance of natural pigments and its positive impact on society, the work plan was designed to synthesize natural pigments from *Pseudomonas* sp.

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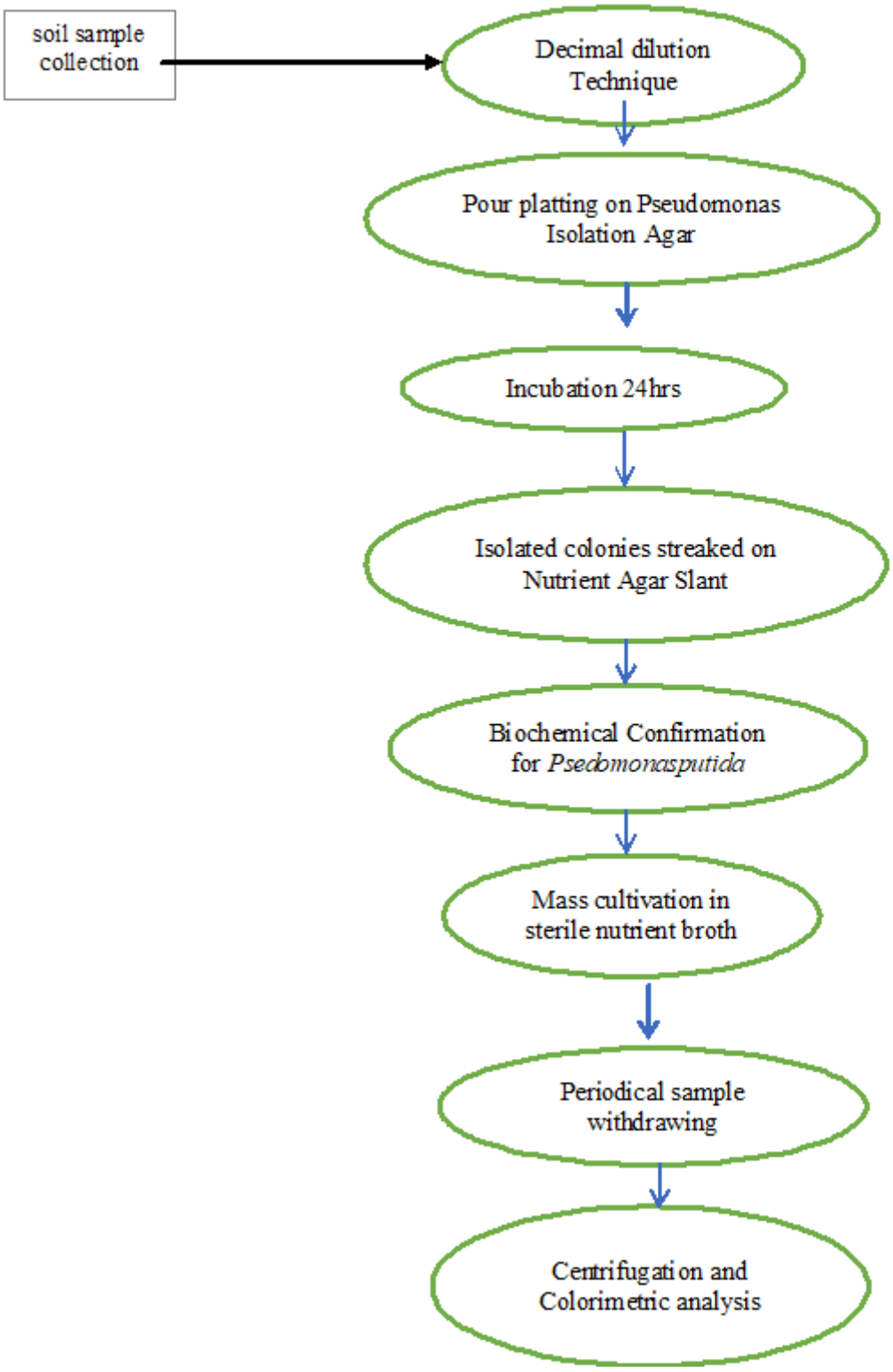
Chart explanation of Plan of Work



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MATERIALS REQUIRED

Collection of sample

Glassware Preparation

All glasswares used were cleaned with 6N HCl to remove residual iron and rinsed with distilled water. All growth media and reagents were prepared in the same water.

The soil samples were collected from the botanical garden of Millerpuram, Thoothukudi, at a depth of 5cm from the substratum. It was collected in sterile containers aseptically and transported immediately to the lab and processed for bacteriological analysis.

Isolation of Bacteria

The collected sample was serially diluted from 10^{-2} to 10^{-9} and the diluted samples were plated on both nutrient and Pseudomonas Isolation Agar. The plates were incubated at 30^0C for 24hrs. After incubation, the total Bacterial Count from Nutrient Agar plate and the individual colony from Pseudomonas Isolation Agar (PSI) were screened for pigment production.

Identification of Bacteria

The selected strain was streaked on nutrient agar slant and stored at 4^0C for further analysis. The bacterial cultures on PSI are identified based on the morphological and biochemical characteristics outlined by **Kanner** et al., (1978).

Purification & Mass cultivation of *P.putida*

After biochemical confirmation, stored *Pseudomonas* culture was quadrant streaked on Nutrient agar plate to check its purity. Well isolated purified colonies were stored for experimental purposes. The mass cultivation has done by preparing 100 ml nutrient broth and was sterilized at 121 lbs for 15minutes followed by the cooling of the medium. The stored *P.putida*was inoculated aseptically into the sterile nutrient broth.

Analysis of the pigment production

An incubation period of 15 days was provided. But once every two days, the sample was collected, centrifuged at 8000 rpm for 15 min and both the supernatant and bacterial cell pellets were collected. Bacterial pellets were then extracted using either 95% (v/v) methanol or 99% (v/v) acetone in the ratio of 1:5 until the pellet was colorless, i.e., complete pigment extraction was achieved and then the pellet was discarded. The pigment extract was then analyzed by scanning the absorbance in the wavelength region of 580nm using a colorimeter. This was performed for 14days and results were collected in triplicate (Kamla*et al*., 2012).

RESULT

During the incubation of *P.putida* in nutrient broth, for the first four days, there was a slight colour change in the media. The colour change was notable from the sixth day onwards. On the eighth day, it was dark green and continued on until the end of the incubation time. The validity of this experiment was demonstrated by colorimetric observation also. The pigment concentration was low (0.089±0.08) during the second day of colorimetric reading. At the time of the sixth and eighth day of colorimetric observation, the concentration of pigments was gradually increased (Fig1). The concentration of pigment production was high during the tenth day of optical density

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observation (0.527±0.16). The colorimetric reading in the subsequent days was more or less the same. The density of the pigments remained intact.

Table 5.1. Biochemical characteristics of *P.putida* employed in this study

Biochemical Tests	***P,putida***
Morphological Tests	
Gram staining	G –ve rods
Motility	Motile
Biochemical characteristics	
Oxidase	+
Catalase	+
Gelatin Hydrolysis	+
Starch Hydrolysis	-
Oxidation-Fermentation	Acid Gas
Florescence	+

Figure 5.2. Pigment Production from *P.putida*

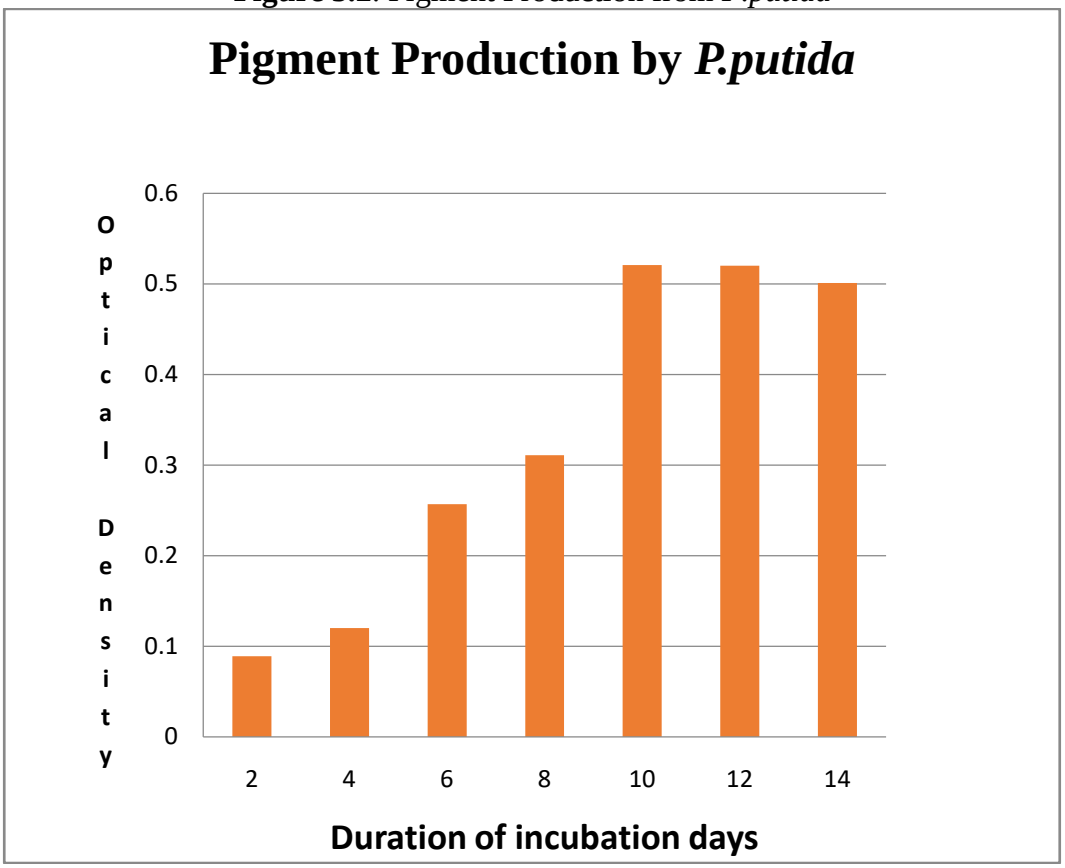

Plate 5.3.3 Isolated colonies of *P.putida*

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Plate 5.3.3 Isolated colonies of *P.putida*

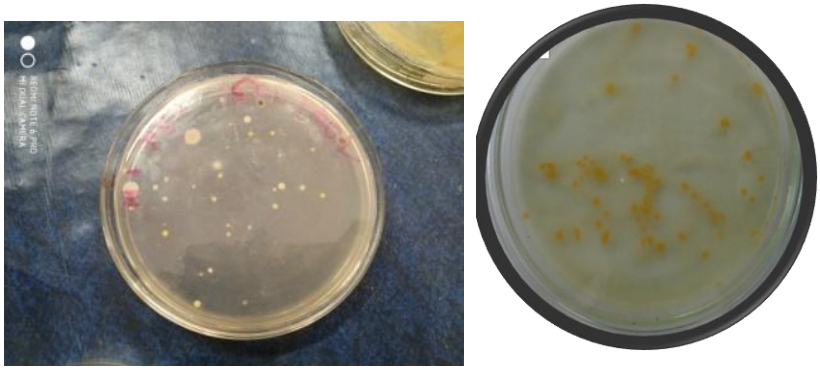

Plate 5.3.4 Pigment production by *P,putida*Plate 5.3.5 Piment production during 10th day ofIncubation

Microbial pigments are the characteristic feature of some bacteria which may be useful in identification. Bacterial pigments offer promising avenues for various applications due to their better biodegradability and higher compatibility with the environment (MeghaWaghela and Shabib Khan, 2018). The present experiment revealed that the microorganism *P.putida* isolated from the soil produces a large amount of pigments.

The advantages of pigment production from microorganisms comprise easy and fast growth in cheap culture medium, independent from weather conditions and colors of different shades (Sinha*et al.*, 2017). According to the above assertion, the simple, easiest nutrient broth medium was used for the pigment producing *P.putida* respectively.

There is growing interest in microbial pigments due to their natural character, safety to use, medicinal properties, nutrients like vitamins, production is independent of season and geographical conditions, and controllable and predictable yield (Nakashima *et al*., 2005).Again bacterial pigments can be produced from waste material thus environmental pollution can be minimized (Joshi *et al*., 2003; Kamla*et al*., 2012).

In the present work, the periodical colorimetric observation of pigment production revealed that the highest production or colour change occurred on the 10th day of incubation and very low pigment production on 2nd day of incubation. After 10th day, not much notable increase in the optical density was observed. This in turn indicated that, there was no more pigment production in the medium.

The reason may be, once the nutrient content in the media began to decrease, the bacteria began to produce pigments to save their lives (antagonism) so it is for this reason, that bacteria not producing pigments in its early days began producing pigments in later days when there is food shortage come. Chidambaram *et al*., (2013) clearly mentioned that, *Pseudomonas aeruginosa* produces a variety of extracellular pigments and they are biologically active metabolites that function in microbial competitiveness.

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Data availability: All datasets and statistical report analyses during this study are included in the manuscript.

Ethics Statement: This article does not contain any studies with human participants or animals.

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